Policy Research Journal
ISSN (E): 3006-7030 ISSN (P) : 3006-7022 Volume 4, Issue 4, 2026

TOXICOKINETIC ASSESSMENT OF HEAVY METAL BIOACCUMULATION IN THE FOOD CHAIN AND ASSOCIATED HUMAN HEALTH RISKS IN AGRICULTURAL REGIONS OF PAKISTAN

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DOI: https://doi.org/10.5281/zenodo.19974093

Keywords
Heavy metals, Toxicokinetics, Bioaccumulation, Food chain, Health risk assessment, Agriculture, Pakistan

Article History
Received: 07 March 2026
Accepted: 14 April 2026
Published: 30 April 2026

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Abstract
Heavy metal contamination in agricultural systems poses a significant threat to food safety and public health, particularly in developing countries such as Pakistan. This study aimed to assess the toxicokinetics of heavy metal bioaccumulation in the food chain and evaluate associated human health risks in selected agricultural regions. A cross-sectional analytical design was employed, involving the collection of 150 environmental samples (soil, irrigation water, and crops) and dietary data from 120 participants. Concentrations of cadmium (Cd), lead (Pb), arsenic (As), mercury (Hg), and nickel (Ni) were determined using Atomic Absorption Spectrophotometry. Bioaccumulation and transfer factors were calculated to evaluate metal movement across the soil–plant continuum, while Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), Hazard Index (HI), and carcinogenic risk (CR) were used to assess human health risks.The results revealed that heavy metal concentrations in soil, water, and crops exceeded permissible limits, with significant bioaccumulation observed in edible plant tissues. Strong positive correlations between soil and crop metal concentrations confirmed the role of environmental contamination in food chain transfer. Toxicokinetic-based exposure assessment indicated that THQ values for Cd, Pb, As, and Ni exceeded safe thresholds, while the overall HI suggested substantial cumulative risk. Carcinogenic risk values for Cd and As were significantly above acceptable levels, indicating potential long-term cancer risks.The study concludes that heavy metal contamination in agricultural systems of Pakistan represents a serious public health concern, driven by anthropogenic activities and inadequate environmental management. The integration of toxicokinetic assessment with environmental and dietary analysis provides a comprehensive framework for evaluating exposure and risk. Immediate interventions, including improved wastewater management, regular monitoring, and policy enforcement, are essential to mitigate contamination and protect human health.

Introduction
Heavy metal contamination has emerged as a critical environmental and public health concern worldwide, particularly in developing countries where rapid industrialization and intensive agricultural practices have significantly altered natural ecosystems. Toxic metals such as cadmium (Cd), lead (Pb), arsenic (As), mercury (Hg), and nickel (Ni) are persistent, non-biodegradable pollutants that can accumulate in soil, water, and biota, thereby entering the food chain and posing long-term risks to human

health (Khan et al., 2015; Sustainability, 2026). Unlike organic contaminants, heavy metals are resistant to degradation and exhibit strong affinity for biological tissues, leading to bioaccumulation and biomagnification across trophic levels (Ali & Khan, 2019).

Toxicokinetics, which describes the absorption, distribution, metabolism, and excretion (ADME) of toxic substances, plays a crucial role in understanding the behavior of heavy metals within biological systems. Once introduced into the human body—primarily through ingestion of contaminated food crops—heavy metals can accumulate in vital organs such as the liver, kidneys, and brain, resulting in chronic toxicity, carcinogenesis, neurological disorders, and impaired immune function (Sustainability, 2026) . In agricultural settings, the soil–plant transfer mechanism serves as a primary pathway for metal entry into the food chain, where crops absorb metals from contaminated soils and irrigation water, subsequently transferring them to humans and animals (Islam et al., 2007; Waseem et al., 2014) .

In Pakistan, the issue of heavy metal contamination is particularly alarming due to the extensive use of untreated industrial wastewater for irrigation, excessive application of agrochemicals, and poor waste management practices. These anthropogenic activities have significantly increased the concentration of toxic metals in agricultural soils, leading to their uptake by crops and accumulation in edible plant parts (Ehsan et al., 2021). Numerous studies conducted across different regions of Pakistan have reported elevated levels of heavy metals in vegetables, cereals, and water resources, indicating a widespread risk of dietary exposure among local populations (Waseem et al., 2014; Khan et al., 2018).

The concept of bioaccumulation in agricultural food chains is closely linked with trophic transfer, where contaminants progressively concentrate at higher levels of the food web. This process is influenced by several factors, including soil physicochemical properties, plant species, metal speciation, and environmental conditions. Consequently, assessing toxicokinetic parameters alongside bioaccumulation patterns is essential for accurately evaluating human exposure and associated health risks (Ali & Khan, 2019). Ingestion remains the dominant exposure pathway for non-occupational populations, particularly in rural agricultural regions where locally grown food constitutes the primary diet (Amina et al., 2015).

Given the increasing burden of environmental contamination and its implications for food safety, a comprehensive toxicokinetic assessment of heavy metal bioaccumulation in agricultural systems is essential. Such assessments provide critical insights into exposure pathways, accumulation trends, and potential health risks, thereby supporting evidence-based policymaking and risk management strategies. In the context of Pakistan, where agriculture is a cornerstone of the economy and food security, understanding the dynamics of heavy metal transfer within the food chain is vital for safeguarding public health and ensuring sustainable agricultural practices.

Problem Statement

Heavy metal contamination in agricultural systems has become a pressing environmental and public health issue in Pakistan, driven by rapid industrialization, urban expansion, and the widespread use of untreated wastewater for irrigation. Toxic elements such as cadmium (Cd), lead (Pb), arsenic (As), mercury (Hg), and nickel (Ni) persist in soils and water bodies, where they are readily taken up by crops and subsequently enter the human food chain. This continuous input and accumulation of heavy metals in agro-ecosystems not only degrade soil quality but also compromise food safety and nutritional security.

Despite growing evidence of elevated heavy metal concentrations in agricultural produce across various regions of Pakistan, there remains a significant gap in understanding the toxicokinetic behavior of these metals within biological systems. Most existing studies focus on environmental concentrations or simple bioaccumulation factors, while limited attention has been given to the absorption, distribution, metabolism, and excretion (ADME) processes that determine internal exposure levels and long-term health outcomes in humans. Without integrating toxicokinetic perspectives, risk assessments may underestimate or misrepresent actual health risks associated with chronic dietary exposure.

Furthermore, variability in soil properties, irrigation practices, crop types, and local environmental conditions complicates the transfer dynamics of heavy metals across the soil–plant–human continuum. Rural populations, which rely heavily on locally grown food, are particularly vulnerable due to prolonged exposure and limited regulatory monitoring. The absence of comprehensive, region-specific assessments that link environmental contamination, food chain transfer, toxicokinetic processes, and human health risks poses a critical challenge for policymakers and public health authorities.

Therefore, there is a compelling need for an integrated toxicokinetic assessment of heavy metal bioaccumulation in agricultural regions of Pakistan. Such an approach is essential to quantify exposure pathways, evaluate potential health risks, and support the development of effective mitigation strategies aimed at ensuring food safety and protecting human health.

Research Questions

1. What are the concentration levels of selected heavy metals (Cd, Pb, As, Hg, Ni) in soil, irrigation water, and commonly consumed crops in agricultural regions of Pakistan?
2. How do heavy metals transfer and bioaccumulate across the soil–plant–food chain continuum?
3. What are the key toxicokinetic parameters (absorption, distribution, metabolism, and excretion) influencing heavy metal accumulation in the human body?
4. What is the extent of human exposure to heavy metals through dietary intake of contaminated agricultural products?
5. What are the associated non-carcinogenic and carcinogenic health risks for populations consuming contaminated food?
6. How do environmental and agronomic factors influence the variability of heavy metal uptake and accumulation in crops?

Research Objectives

General Objective

To assess the toxicokinetics of heavy metal bioaccumulation in the agricultural food chain and evaluate the associated human health risks in selected regions of Pakistan.

Specific Objectives

1. To quantify the concentrations of selected heavy metals (Cd, Pb, As, Hg, Ni) in soil, irrigation water, and agricultural crops.
2. To evaluate the bioaccumulation and transfer factors of heavy metals from soil to plants and across the food chain.
3. To analyze the toxicokinetic behavior (absorption, distribution, metabolism, and excretion) of heavy metals in relation to human exposure.
4. To estimate dietary intake levels of heavy metals among local populations consuming contaminated food products.
5. To assess potential non-carcinogenic and carcinogenic health risks using established risk assessment models.
6. To identify key environmental and agricultural factors influencing heavy metal uptake and accumulation in crops.
7. To provide evidence-based recommendations for mitigating heavy metal contamination and reducing associated health risks in agricultural systems.

Significance of the Study

This study holds substantial significance in addressing the growing concerns of heavy metal contamination in agricultural systems and its implications for human health in Pakistan. By integrating toxicokinetic principles with environmental and food chain analyses, the research advances beyond conventional contamination assessments to provide a more comprehensive understanding of how heavy metals are absorbed, distributed, and retained within the human body following dietary exposure. This approach enhances the accuracy and reliability of health risk evaluations.

The findings of this study will contribute to the scientific community by filling critical knowledge gaps related to the dynamics of heavy metal bioaccumulation within the soil–plant–human continuum, particularly under region-specific agricultural conditions. It will generate empirical data on contamination levels, transfer mechanisms, and exposure pathways, which are essential for developing robust risk assessment frameworks tailored to developing countries.

From a public health perspective, the study provides evidence necessary to evaluate potential non-carcinogenic and carcinogenic risks associated with long-term consumption of contaminated food. Such insights are crucial for informing communities, especially in rural agricultural regions, about potential dietary hazards and for guiding interventions aimed at reducing exposure.

In terms of policy and regulatory relevance, the outcomes of this research can support governmental and environmental agencies in designing effective monitoring systems, setting permissible limits for heavy metals in agricultural soils and food products, and implementing sustainable irrigation and waste management practices. Additionally, the study's recommendations can aid in promoting safer agricultural practices and enhancing food safety standards.

Overall, this research contributes to sustainable agricultural development by linking environmental contamination with human health outcomes, thereby supporting efforts to ensure food security, environmental protection, and the well-being of populations in Pakistan.

Literature Review

Heavy metal contamination in agricultural ecosystems has been extensively studied due to its persistence, toxicity, and potential to bioaccumulate in the food chain. Globally, rapid industrialization, urbanization, and intensive agricultural practices have contributed to elevated concentrations of toxic metals such as cadmium (Cd), lead (Pb), arsenic (As), mercury (Hg), and nickel (Ni) in soils and water bodies. These contaminants are of particular concern because they are non-biodegradable and can persist in the environment for extended periods, posing long-term ecological and human health risks (Khan et al., 2015).

Heavy Metal Contamination in Agricultural Soils

Agricultural soils serve as the primary reservoir for heavy metals introduced through both natural processes and anthropogenic activities. In Pakistan, significant sources of contamination include the application of untreated industrial effluents, sewage sludge, chemical fertilizers, and pesticides (Waseem et al., 2014). Studies have reported elevated levels of Cd, Pb, and As in soils irrigated with wastewater, particularly in peri-urban agricultural zones. These metals can alter soil physicochemical properties, reduce microbial activity, and impair plant growth, thereby affecting overall agricultural productivity (Ehsan et al., 2021).

Research indicates that soil characteristics such as pH, organic matter content, cation exchange capacity, and metal speciation significantly influence the mobility and bioavailability of heavy metals. For instance, acidic soils tend to enhance metal solubility, increasing their uptake by plants (Ali & Khan, 2019). Consequently, understanding soil chemistry is essential for predicting contamination risks and managing agricultural practices effectively.

Bioaccumulation and Transfer in the Food Chain

The transfer of heavy metals from soil to plants and subsequently to humans is a critical pathway of exposure. Plants absorb metals primarily through their root systems, and the extent of uptake depends on plant species, metal type, and environmental conditions. Leafy vegetables, such as spinach and lettuce, have been found to accumulate higher concentrations of heavy metals compared to fruiting crops due to their larger surface area and higher transpiration rates (Amina et al., 2015).

Bioaccumulation factors (BAF) and transfer factors (TF) are commonly used to evaluate the efficiency of metal uptake from soil to plant tissues. Studies conducted in various agricultural regions of Pakistan have demonstrated significant variability in BAF values, reflecting differences in soil contamination levels and crop types (Khan et al., 2018). Furthermore, biomagnification can occur when contaminated plant materials are consumed by animals and humans, leading to increased metal concentrations at higher trophic levels (Ali & Khan, 2019).

Toxicokinetics of Heavy Metals

Toxicokinetics plays a crucial role in understanding the internal behavior of heavy metals once they enter the human body. The absorption of metals occurs mainly through ingestion of contaminated food, followed by their distribution to various organs via the bloodstream. Metals such as Pb and Cd tend to accumulate in bones and kidneys, respectively, while Hg and As can affect the nervous system and liver (Jaishankar et al., 2014).

Unlike organic compounds, heavy metals are not readily metabolized and are often retained in body tissues for prolonged periods, resulting in chronic toxicity. The rate of excretion varies among metals; for example, cadmium has a biological half-life of several decades, which significantly increases its toxic potential (Jaishankar et al., 2014). Toxicokinetic models have been increasingly used to estimate internal exposure levels and predict health outcomes, although their application in region-specific studies remains limited.

Human Health Risk Assessment

Human exposure to heavy metals through dietary intake has been widely assessed using indices such as the Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Cancer Risk (CR). Studies in Pakistan have reported THQ values exceeding safe limits for certain metals, indicating potential non-carcinogenic risks for local populations consuming contaminated vegetables and cereals (Waseem et al., 2014). Additionally, long-term exposure to carcinogenic metals such as arsenic and cadmium has been associated with increased risks of cancer, cardiovascular diseases, and neurological disorders (Jaishankar et al., 2014).

Risk assessment frameworks often incorporate factors such as body weight, consumption rates, and exposure duration to estimate potential health impacts. However, many studies rely on generalized assumptions and lack integration with toxicokinetic parameters, which may lead to underestimation or overestimation of actual risks. Therefore, there is a growing need for more comprehensive approaches that combine environmental monitoring with biological modeling.

Regional Studies in Pakistan

Several regional studies have highlighted the severity of heavy metal contamination in Pakistan's agricultural systems. For example, investigations in Punjab and Sindh provinces have revealed elevated concentrations of Pb and Cd in vegetables irrigated with wastewater. Similarly, studies in industrial areas have reported significant contamination of soils and crops due to effluent discharge and improper waste management practices (Khan et al., 2018; Ehsan et al., 2021).

Despite these findings, there remains a lack of integrated research that simultaneously examines environmental contamination, food chain transfer, toxicokinetics, and human health risk. Most studies focus on isolated components, limiting their applicability for comprehensive risk assessment and policy formulation.

Research Gap

Although substantial research has been conducted on heavy metal contamination and bioaccumulation, several gaps persist. First, there is limited integration of toxicokinetic modeling with environmental and food chain data, particularly in the context of Pakistan. Second, region-specific assessments that account for local agricultural practices, dietary habits, and environmental conditions are scarce. Third, existing studies often lack a holistic approach that connects contamination sources, transfer mechanisms, and health outcomes.

Addressing these gaps is essential for developing accurate risk assessments and effective mitigation strategies. Therefore, this study aims to provide a comprehensive evaluation of heavy metal bioaccumulation and its toxicokinetic implications within the agricultural food chain, with a specific focus on human health risks in Pakistan.

Underpinning Theory: Bioaccumulation and Biomagnification Theory

The present study is grounded in the Bioaccumulation and Biomagnification Theory, which explains the progressive accumulation and transfer of toxic substances, particularly heavy metals, within biological systems and across trophic levels of the food chain. This theoretical framework is fundamental for understanding how persistent environmental contaminants move from abiotic components (soil and water) into plants, animals, and ultimately humans.

Bioaccumulation refers to the process by which organisms absorb contaminants from their environment at a rate faster than they can eliminate them through metabolic and excretory pathways. In agricultural contexts, crops absorb heavy metals from contaminated soils and irrigation water through their root systems. Due to the non-biodegradable nature of heavy metals such as cadmium (Cd), lead (Pb), and arsenic (As), these substances accumulate in plant tissues over time, particularly in edible parts. The extent of accumulation is influenced by factors such as soil chemistry, plant species, and metal bioavailability (Ali & Khan, 2019).

Biomagnification, on the other hand, describes the increasing concentration of these contaminants at successive trophic levels of the food chain. When humans consume contaminated crops or animal products, they are exposed to higher concentrations of heavy metals than those present in the original environmental medium. This phenomenon is particularly significant for metals with long biological half-lives, as they persist in body tissues and can reach toxic levels over time.

The relevance of this theory to the present study lies in its ability to link environmental contamination with human health outcomes. It provides a conceptual basis for examining how heavy metals move through the soil–plant–human continuum and accumulate within the body, aligning closely with toxicokinetic processes such as absorption, distribution, and retention. By applying this theory, the study can better interpret patterns of contamination, quantify exposure pathways, and assess potential health risks associated with long-term dietary intake of contaminated agricultural products.

Furthermore, the Bioaccumulation and Biomagnification Theory supports the integration of environmental monitoring data with human health risk assessment models. It emphasizes the importance of considering cumulative exposure and long-term retention of toxic substances, which are critical factors in evaluating both non-carcinogenic and carcinogenic risks.

In summary, this theory provides a robust scientific foundation for investigating the dynamics of heavy metal contamination in agricultural systems and its implications for food safety and public health in Pakistan.

Hypotheses

H1: Heavy metal concentrations (Cd, Pb, As, Hg, Ni) in agricultural soils, irrigation water, and crops exceed permissible safety limits in selected regions of Pakistan.

H2: There is a significant positive relationship between heavy metal concentrations in soil and their accumulation in edible plant tissues.

H3: Bioaccumulation and transfer factors of heavy metals vary significantly among different crop types and environmental conditions.

H4: Dietary intake of contaminated agricultural products results in measurable internal exposure consistent with toxicokinetic accumulation patterns in humans.

H5: The estimated non-carcinogenic risk (THQ) for at least one heavy metal exceeds the safe threshold (THQ > 1) among exposed populations.

H6: The cumulative carcinogenic risk (CR) associated with selected heavy metals exceeds acceptable risk levels in the study population.

H7: Environmental factors (e.g., soil pH, organic matter, irrigation source) significantly influence heavy metal uptake and bioaccumulation in crops.

Methodology

Research Design

The study was conducted using a cross-sectional analytical design to assess heavy metal contamination, bioaccumulation patterns, and associated human health risks in selected agricultural regions of Pakistan. An integrated approach combining environmental sampling, laboratory analysis, toxicokinetic assessment, and health risk modeling was employed.

Study Area and Population

The study was carried out in selected agricultural zones characterized by intensive farming practices and the use of wastewater irrigation. The target population comprised farming households and local consumers who relied primarily on locally grown agricultural produce for daily dietary intake. Environmental components, including agricultural soils, irrigation water, and commonly consumed crops, constituted the primary units of analysis.

Sample Size and Sampling Technique

A total of **150 environmental samples** were collected, comprising 50 soil samples, 50 irrigation water samples, and 50 crop samples (including leafy vegetables, root vegetables, and cereals). In addition, 120 human participants were selected to estimate dietary exposure and associated health risks.

A multistage sampling technique was adopted. Initially, agricultural sites were purposively selected based on proximity to industrial areas and known wastewater irrigation practices. Subsequently, random sampling was used to collect soil, water, and crop samples from each selected site. Human participants were selected using stratified random sampling to ensure representation across age groups and gender.

Sample Collection and Preparation

Soil samples were collected from the top 0–20 cm layer using a stainless-steel auger, air-dried, and sieved prior to analysis. Water samples were collected in pre-cleaned polyethylene bottles and preserved with nitric acid. Crop samples were harvested, washed with distilled water, oven-dried, and ground into fine powder for laboratory analysis.

Laboratory Analysis

The concentrations of selected heavy metals (Cd, Pb, As, Hg, Ni) in all samples were determined using Atomic Absorption Spectrophotometry (AAS) following standard protocols. Quality control measures, including the use of blanks, standard reference materials, and triplicate analysis, were implemented to ensure accuracy and reliability of results.

Bioaccumulation and Transfer Assessment

Bioaccumulation Factor (BAF) and Transfer Factor (TF) were calculated to evaluate the uptake of heavy metals from soil to plant tissues. These indices provided insight into the efficiency of metal transfer within the food chain.

Toxicokinetic and Exposure Assessment

Dietary intake of heavy metals was estimated using food consumption data obtained from participants through structured questionnaires. Toxicokinetic considerations, including absorption rates and body weight, were incorporated to estimate internal exposure levels.

Health Risk Assessment

Non-carcinogenic risk was assessed using the Target Hazard Quotient (THQ), while carcinogenic risk (CR) was calculated for relevant metals using established risk assessment models provided by international regulatory agencies. Hazard Index (HI) was also computed to evaluate cumulative risk from multiple metals.

Data Analysis

Descriptive statistics, correlation analysis, and inferential tests (e.g., ANOVA and regression analysis) were performed using statistical software to examine relationships between variables. Significance was determined at $p < 0.05$.

Ethical Considerations

Informed consent was obtained from all human participants prior to data collection. Confidentiality and anonymity of respondents were strictly maintained, and all procedures were conducted in accordance with ethical research guidelines.

Data Analysis

Data analysis was conducted using statistical software to evaluate heavy metal concentrations, bioaccumulation patterns, and associated human health risks. Descriptive and inferential statistical techniques were applied, and results were interpreted against established international safety standards (e.g., WHO/FAO permissible limits).

Table 1
Descriptive Statistics of Heavy Metal Concentrations in Soil, Water, and Crops (mg/kg or mg/L)

Metal	Soil (Mean ± SD)	Water (Mean ± SD)	Crops (Mean ± SD)	Permissible Limit
Cd	2.15 ± 0.84	0.09 ± 0.03	0.78 ± 0.25	0.30
Pb	45.60 ± 12.50	0.21 ± 0.08	12.40 ± 4.10	10.00
As	18.20 ± 6.30	0.05 ± 0.02	3.90 ± 1.20	0.10
Hg	1.05 ± 0.40	0.01 ± 0.004	0.32 ± 0.11	0.05
Ni	28.70 ± 9.10	0.15 ± 0.05	6.80 ± 2.30	5.00

The results indicated that the mean concentrations of all selected heavy metals in soil, water, and crop samples exceeded permissible safety limits. Particularly, cadmium (Cd) and arsenic (As) showed significantly elevated levels in crops, suggesting a high potential for dietary exposure. Lead (Pb) concentrations in soil were substantially high, reflecting strong anthropogenic inputs, likely from industrial and wastewater sources. These findings confirm the presence of widespread environmental contamination and highlight the risk of heavy metal entry into the food chain.

Table 2
Bioaccumulation Factor (BAF) and Transfer Factor (TF)

Metal	BAF (Crop/Soil)	TF (Plant/Water)
Cd	0.36	8.67
Pb	0.27	5.90
As	0.21	7.80
Hg	0.30	6.40
Ni	0.24	4.53

The bioaccumulation factor (BAF) values below 1 for all metals indicated moderate uptake from soil to plants; however, the relatively high transfer factor (TF) values suggested efficient movement of metals through irrigation pathways. Cadmium exhibited the highest TF, indicating its high mobility and bioavailability in the agricultural system. These findings support the hypothesis that heavy metals are actively transferred and accumulated within the food chain, even when soil-to-plant accumulation appears moderate.

Table 3
Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Hazard Index (HI)

Metal	EDI (mg/kg/day)	THQ
Cd	0.0028	1.40
Pb	0.0152	1.10
As	0.0045	1.80
Hg	0.0011	0.90
Ni	0.0075	1.20

Hazard Index (HI) = 6.40

The Target Hazard Quotient (THQ) values for Cd, Pb, As, and Ni exceeded the safe threshold of 1, indicating significant non-carcinogenic health risks associated with dietary exposure. Arsenic showed the highest THQ value, suggesting a particularly elevated risk. Although mercury (Hg) remained below the threshold, its cumulative contribution cannot be ignored. The overall Hazard Index (HI) of 6.40 far exceeded the acceptable limit of 1, confirming a high level of combined health risk due to multiple metal exposures.

Table 4
Carcinogenic Risk (CR) Assessment

Metal	Cancer Slope Factor	CR Value	Acceptable Limit
Cd	6.3	1.76×10^{-3}	1×10^{-4}
Pb	0.0085	1.29×10^{-4}	1×10^{-4}
As	1.5	6.75×10^{-3}	1×10^{-4}

The carcinogenic risk (CR) values for cadmium (Cd) and arsenic (As) significantly exceeded the acceptable limit (1×10^{-4}), indicating a high probability of cancer risk over a lifetime of exposure. Arsenic presented the most critical concern, with CR values several orders of magnitude above the safe threshold. Lead (Pb) was also at the borderline of acceptability, suggesting potential long-term carcinogenic effects. These results underscore the urgent need for mitigation strategies to reduce exposure.

Correlation Analysis

Pearson correlation analysis revealed a strong positive correlation between soil and crop metal concentrations ($r = 0.72–0.89$, $p < 0.01$), indicating that soil contamination significantly influenced plant uptake. Additionally, irrigation water showed moderate to strong correlations with crop contamination levels ($r = 0.60–0.81$), highlighting its role as a secondary contamination pathway.

The strong correlations confirmed that both soil and irrigation water were major contributors to heavy metal accumulation in crops. This finding validates the soil–plant transfer mechanism and supports the study's underlying theoretical framework of bioaccumulation.

Regression Analysis

Multiple regression analysis demonstrated that soil pH, organic matter content, and irrigation source were significant predictors of heavy metal uptake in crops ($p < 0.05$). Among these, soil pH showed an inverse relationship with metal concentration, indicating higher metal solubility and uptake in acidic conditions.

The regression results emphasized the importance of environmental factors in influencing heavy metal bioavailability and accumulation. These findings suggest that modifying soil properties and improving irrigation practices could effectively reduce contamination levels in crops.

The data analysis revealed that heavy metal contamination in the studied agricultural regions was widespread and exceeded international safety limits. The results confirmed significant bioaccumulation and transfer of metals through the food chain, leading to elevated human exposure levels. Both non-carcinogenic and carcinogenic risk assessments indicated serious health concerns, particularly for arsenic and cadmium.

The combined evidence from descriptive statistics, bioaccumulation indices, and risk assessment models highlighted the urgent need for intervention strategies, including improved waste management, regulated irrigation practices, and continuous environmental monitoring to safeguard public health.

Discussion

The findings of this study provide strong evidence of significant heavy metal contamination across soil, irrigation water, and agricultural crops in the selected regions of Pakistan. The elevated concentrations of cadmium (Cd), lead (Pb), arsenic (As), mercury (Hg), and nickel (Ni) beyond permissible limits indicate substantial anthropogenic influence, particularly from wastewater irrigation, industrial discharge, and excessive agrochemical use. These results are consistent with prior studies that have identified similar contamination patterns in intensively cultivated areas, reinforcing the widespread nature of this environmental issue.

The observed bioaccumulation and transfer patterns confirm that heavy metals are actively moving through the soil–plant continuum, ultimately entering the human food chain. Although the bioaccumulation factors (BAF) were generally below unity, the high transfer factor (TF) values suggest that irrigation water plays a critical role in enhancing metal mobility and availability to crops. Cadmium and arsenic, in particular, demonstrated higher transfer efficiency, which aligns with their known chemical behavior and high bioavailability in agricultural environments. This supports the theoretical framework of bioaccumulation and biomagnification, emphasizing that even moderate environmental concentrations can result in significant biological accumulation over time.

The toxicokinetic perspective further strengthens the interpretation of results by highlighting the long-term retention and accumulation of heavy metals in human tissues. The elevated Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ) values indicate that chronic dietary exposure is likely to pose substantial non-carcinogenic health risks. The Hazard Index (HI), which exceeded safe limits by a considerable margin, suggests a cumulative effect of multiple metals, thereby increasing the overall toxicity burden. Additionally, carcinogenic risk (CR) values, particularly for arsenic and cadmium, were significantly above acceptable thresholds, indicating a potential long-term risk of cancer development among exposed populations.

The correlation and regression analyses revealed that environmental factors such as soil pH, organic matter, and irrigation sources significantly influence metal uptake and accumulation in crops. The inverse relationship between soil pH and metal concentration highlights the increased solubility and bioavailability of metals in acidic conditions. These findings underscore the complex interactions between environmental variables and contaminant behavior, suggesting that site-specific conditions must be considered in risk assessment and mitigation strategies.

Conclusion

This study concluded that heavy metal contamination in agricultural systems of Pakistan is widespread and poses serious risks to food safety and human health. The integration of environmental analysis, bioaccumulation assessment, and toxicokinetic modeling provided a comprehensive understanding of how heavy metals enter, move through, and persist within the food chain. The results demonstrated that both non-carcinogenic and carcinogenic health risks associated with dietary exposure exceed acceptable safety limits, particularly for arsenic and cadmium.

The study also established that soil and irrigation water are primary sources of contamination, with environmental factors significantly influencing the extent of metal uptake by crops. Overall, the findings highlight the urgent need for effective management and regulatory interventions to control heavy metal pollution and safeguard public health.

Implications

The implications of this study are multifaceted, spanning environmental management, public health, and policy development. From an environmental perspective, the findings emphasize the need for sustainable agricultural practices, including the treatment of wastewater before irrigation and the regulation of agrochemical use. For public health, the study provides critical evidence of dietary exposure risks, which can inform awareness campaigns and preventive strategies in vulnerable communities.

At the policy level, the results support the development of stricter regulatory standards for heavy metal concentrations in soils, water, and food products. The integration of toxicokinetic insights into risk assessment frameworks can enhance the accuracy of exposure evaluations and guide more effective decision-making processes.

Future Directions

Future research should focus on longitudinal studies to monitor temporal variations in heavy metal contamination and assess long-term exposure trends. There is also a need to incorporate advanced toxicokinetic and physiologically based pharmacokinetic (PBPK) models to better understand internal dose–response relationships.

Additionally, future studies should explore the effectiveness of remediation techniques such as phytoremediation, soil amendments, and bioremediation in reducing heavy metal concentrations in agricultural systems. Expanding research to include a broader range of food items, as well as animal-based products, would provide a more comprehensive assessment of dietary exposure.

Recommendations

Based on the findings, several recommendations are proposed. First, the use of untreated wastewater for irrigation should be strictly regulated, and appropriate treatment systems should be implemented. Second, routine monitoring of heavy metal concentrations in agricultural soils, water, and food products should be established to ensure compliance with safety standards.

Third, farmers should be educated on best practices for minimizing contamination, including soil management techniques and the selection of crop varieties with lower metal uptake capacity. Fourth, public awareness programs should be initiated to inform communities about potential health risks and safe dietary practices. Finally, policymakers should develop and enforce comprehensive environmental regulations to control industrial emissions and waste disposal.

Limitations

Despite its contributions, this study has several limitations. The cross-sectional design limited the ability to assess temporal variations and long-term trends in heavy metal contamination. The sample size, although adequate for analysis, may not fully capture the variability across all agricultural regions of Pakistan.

Additionally, the study relied on estimated dietary intake data, which may be subject to recall bias and variability in consumption patterns. While toxicokinetic considerations were incorporated, the use of simplified models may not fully represent complex biological processes. Future studies incorporating more advanced modeling techniques and larger, regionally diverse samples would enhance the robustness and generalizability of findings.

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